Effect of Prebiotic on Gut Development and Ascites Incidence of Broilers Reared in a Hypoxic Environment[1]

F. Solis de los Santos,† M. B. Farnell,* G. Téllez,† J. M. Balog,* N. B. Anthony,† A. Torres-Rodriguez,† S. Higgins,† B. M. Hargis,† and A. M. Donoghue*,[2]

*Poultry Production and Product Safety Research Unit, Agricultural Research Service, United States Department of Agriculture, Fayetteville, Arkansas 72701; and †Department of Poultry Science, University of Arkansas, Fayetteville, Arkansas 72701

ABSTRACT Modern broilers have been genetically selected for an increased growth rate and improved feed conversion, but they are also more susceptible to ascites. Ascites occurs when there is an imbalance between available oxygen and the oxygen demand of the broiler. We hypothesized that promoting neonatal gut development with a prebiotic, such as *Aspergillus* meal (Prebiotic-AM), would enhance gut efficiency, decrease the oxygen demand of the gut, and reduce ascites incidence. In this study, we compared the effect of Prebiotic-AM on ascites incidence and gut development in commercial broilers reared at a local altitude (390 m above sea level) and a simulated high altitude (2,900 m above sea level). Half of the birds received a National Research Council recommended corn-soybean ration, and the other half received the same ration supplemented with 0.2% Prebiotic-AM. These 2 groups were further divided into a local altitude group and a simulated high altitude group for a total of 4 treatment combinations. Tissues were collected on d 1, 3, 7, 14, and 21 from the duodenum and lower ileum and placed in 10% buffered formalin for morphometric analysis. At a simulated high altitude, ascites incidence was 68% for birds fed the Prebiotic-AM supplement compared with 92% ascites incidence in birds given the control feed. The simulated high altitude decreased ($P < 0.05$) gut development, but prebiotic-treated birds reared in hypoxic conditions had similar gut development to control birds reared at local altitude. These data suggest that a feed ration supplemented with Prebiotic-AM may reduce the effect of hypoxia on broiler gut development and ascites incidence.

(*Key words*: ascites, gut development, prebiotic, hypoxia, chicken)

2005 Poultry Science 84:1092–1100

INTRODUCTION

The modern broiler has been intensely selected for higher growth rates and increased feed conversion (Pakdel et al., 2002). Broilers from the 1950s required 14 wk to reach market body weight, whereas birds today are ready for market at 6 wk of age with a body weight of 2.6 kg (Havenstein et al., 1994). Unfortunately, lung capacity does not always meet the oxygen demand necessary for rapid growth. If the lung of the chicken grows less rapidly than the rest of the body, hypoxia and ascites could result (Julian, 2000). Ascites is a metabolic disease characterized by an accumulation of fluid in the abdominal cavity, hypertrophy of the right ventricle, and a flaccid heart (Riddell, 1991). High incidence of ascites can occur when broilers are reared at altitudes high enough to substantially reduce the partial pressure of oxygen (Owen et al., 1990; Wideman et al., 2003). It is estimated that 5% of broilers and 20% of roaster birds die of ascites (see review, Balog, 2003); considering that an estimated 40 billion broilers are produced annually around the world, it is evident that the economic losses due to ascites are significant.

The gastrointestinal tract (GIT) is a highly metabolically active organ that has considerable nutrient and oxygen requirements (Yen et al., 1989). The GIT and cardiopulmonary system are dependent upon each other, but the relationship can be negatively influenced by inflammation, pathogens, environment, or a high metabolism resulting in ascites (Ivatury et al., 1996). The high oxygen demand of the gut on the heart and lungs may explain why feed restriction can reduce ascites incidence in broilers; however, decreasing feed consumption can also decrease pro-

Received for publication December 13, 2004.

Accepted for publication March 14, 2005.

[1]Mention of a trade name, proprietary product, or specific equipment does not constitute a guarantee or warranty by the USDA and does not imply its approval to the exclusion of other products that may be suitable.

[2]To whom correspondence should be addressed: donoghue@uark.edu.

Abbreviation Key: GIT = gastrointestinal tract; Prebiotic-AM = *Aspergillus* meal.

ductivity (Balog et al., 2000). Although the total oxygen demand of the gut is not known for poultry; it has been determined in other monogastric animals such as pigs. The pig GIT (including the spleen and pancreas) represents only 5% of the total body weight; however, it consumes 25% of the total oxygen (Yen et al., 1989).

In chicks, the GIT develops rapidly during the first few days post hatch due to a transition in nutrient sources that occurs 3 to 5 d of age when the GIT switches from utilizing the lipid-rich yolk in the yolk sac to an exogenous feed ration that is rich in carbohydrates (Uni et al., 1999). During the first week post hatch, the weight of the small intestine increases faster than the overall body weight (Uni et al., 2003). Early feeding during this period improves initial gut development and provides long-term increases in feed efficiency in market age birds (Uni and Ferket, 2004). Alternatively, chicks that are not provided immediate access to water and feed have an immature GIT, which may result in stunted growth, reduced disease resistance, inefficient feed utilization, and poor meat yield when the bird reaches market age (Uni and Ferket, 2004).

In addition to early or in ovo nutrition, researchers are investigating feed additives that may stimulate early gut development and improve overall efficiency of the chicken GIT. Diet, enzymes, antimicrobials, normal microflora, pathogens, probiotics, and prebiotics directly affect development of the gut and have been manipulated to achieve increased feed conversion and pathogen reduction (Bedford, 2000a; Gilmore and Ferretti, 2003; Apajalahti et al., 2004). Beneficial bacteria, such as *Lactobacilli* and *Bifidobacteria*, can enhance the metabolism of host birds and improve gut efficiency by increasing nutrient absorption (Yokota and Coates, 1982) and accelerating gut development (Palmer and Rolls, 1983; Furese et al., 1991). Prebiotics are nondigestible food ingredients that can selectively stimulate the growth of endogenous bacteria, such as *Lactobacilli* and *Bifidobacteria*, which benefit the host (Gibson et al., 1995). We proposed that the addition of the prebiotic, *Aspergillus* meal (Prebiotic-AM), to broiler feed rations improves gut development in neonates and that the increased enteric efficiency might reduce oxygen demands by the GIT alleviating factors leading to ascites. The effects of Prebiotic-AM on gut development and ascites incidence in commercial broilers reared under normal and hypoxic conditions were evaluated in this study.

MATERIALS AND METHODS

Housing

Birds were placed in stainless-steel battery units housed in environmentally matched chambers at a local altitude or one that simulated a high altitude, low oxygen environment by creating a partial vacuum (Balog et al., 2000). Both chambers measured 3.7 × 2.4 m and were matched in terms of temperature and ventilation. Daily management was conducted under a partial vacuum through the use of an airlock that allowed for pressure equilibration.

Experimental Design

Two hundred six commercial broiler chicks were placed at 1 d of age in the hypobaric chamber simulating 2,900 m above sea level or at 390 m above sea level (local altitude). Chicks at each altitude were further divided into 2 groups: a control group fed a corn-soybean broiler diet ad libitum formulated without antibiotics or coccidiostats that met or exceeded levels of critical nutrients recommended by the National Research Council (1994) and a prebiotic diet (Prebiotic-AM) consisting of the same ingredients as the control but supplemented with 0.2% of *Aspergillus* meal.[3] Birds within treatments were further divided into 2 groups: one to assess ascites incidence over the course of the study and the second to assess gastrointestinal morphology at different ages throughout the study. To detect ascites incidence, 4 replicates of 6 birds per each diet treatment were evaluated for the hypobaric altitude group (a total of 24 birds/treatment), and 4 replicates of 5 birds each per diet treatment were evaluated for the local altitude group (a total of 20 birds/treatment). Extra birds were included in the hypoxic groups to account for expected high ascites-related mortalities and the minimal loss of birds at local altitude observed in previous studies (Balog et al., 2000). The remaining birds were housed together by treatment and altitude, and 4 birds/treatment per altitude per day were randomly selected for evaluation of gastrointestinal morphology. Samples were collected on d 1, 3, 7, 14, and 21 from nonascitic birds (4 treatments × 4 birds × 5 d = 80 birds). The remaining birds were mortalities during the study, had ascites as determined by fluid accumulation in the peritoneum and therefore not used for enteric evaluation, or were healthy birds but not needed for evaluation by d 21.

Ascites Incidence

Ascites incidence was characterized by heart enlargement and fluid accumulation in the peritoneum, as previously described by Balog and coworkers (2003). Birds were checked twice daily to record mortality and to determine if the mortalities were due to ascites. At the end of the study, all surviving birds were euthanized and evaluated for the presence of ascites. Total ascites incidence was determined by combining ascites mortality data throughout the study and the ascites incidence present on the last day of the experiment. Body weight and intestinal weight (dissected from below the gizzard to the ileal cecal junction) were determined on each collection day and on a weekly basis after d 21.

Morphometric Analysis of the Gut

For enteric morphometric analysis, birds on the designated evaluation day were euthanized, and small intes-

[3]PetAg Inc., Hampshire, IL.

tines were collected. A 1-cm segment of the midpoint of the duodenum and the distal end of the lower ileum were removed and fixed in 10% buffered formalin for 72 h. Each segment was then embedded in paraffin, and a 2-μm section of each sample was placed on a glass slide and stained with hematoxylin and eosin for examination with a light microscope (Sakamoto et al., 2000). The parameters evaluated were villus height, villus base, villus surface area, lamina propria thickness, and crypt depth. Morphological parameters were measured using the Image Pro Plus v 4.5 software package.[4] Fourteen measurements were taken per bird per parameter. Villus height was measured from the top of the villus to the top of the lamina propria. Villus surface area was calculated using the formula $(2\pi)(VW/2)(VL)$, where VW = villus width, and VL = villus length (Sakamoto et al., 2000). The lamina propria thickness was measured from the base of the villus to the top of the muscularis mucosa. Crypt depth was measured from the base upwards to the region of transition between the crypt and villus (Aptekmann et al., 2001).

Statistical Analysis

All percentage data were subjected to arc sine transformation. The experimental design used a 2 × 2 factorial arrangement of treatments. Ascites incidence and gut morphology data were subjected to ANOVA using SAS software (SAS Institute, 1988). Mean separation was accomplished using Duncan's multiple range test (Duncan, 1955). A probability value of less than 0.05 was considered significant.

RESULTS

Ascites Incidence and Mortality

At 4 wk of age, ascites was observed in the majority of birds reared at a simulated high altitude; with no birds reared at the local altitude groups showing signs of disease. At high altitude, ascites incidence was lower (68%) in the Prebiotic-AM group compared with controls (92%; Table 1) reared in the same environment, at the completion of the study (d 42).

Body and Gut Weights

Body and gut weights were not different for the diet treatments across altitudes on d 1 to 7; however, on d 14 gut weight for controls reared at a local altitude were greater than the hypoxic controls, and body weight was lower for the hypoxic-treated Prebiotic-AM group compared with the local altitude group ($P < 0.05$, Table 2). There was no difference in body and gut weight between control and prebiotic-treated birds on d 1 to 14 or 28 to 42 within altitudes (Table 2). However, on d 21 body and gut weights were lower in the Prebiotic-AM group reared under hypoxic conditions when compared with the control group ($P < 0.05$, Table 2).

TABLE 1. Effect of altitude and prebiotic on commulative ascites incidence of commercial chicks reared at a local altitude or in a hypoxic environment[1]

	Local (%)		Hypoxic (%)	
Week	Control	Prebiotic	Control	Prebiotic
1	0	0	0	0
2	0	0	0	8.35
3	0	0	29.18[a]	32.15[a]
4	0	0	62.48[a]	44.05[a]
5	0	0	66.65[a]	55.95[a]
6	0	0	87.50[a]	64.30[b]
At necropsy	0	0	4.18[a]	4.18[a]
Total ascites[2]	0	0	91.68[a]	68.48[b]

[a,b]Significant ($P < 0.05$) between treatments within an altitude.

[1]n = 20 birds per diet treatment (local altitude), and n = 24 birds per diet treatment (hypoxic altitude).

[2]Cumulative ascites over a 6-wk period plus ascites found at the final necropsy.

Duodenum Villi Height

Duodenum villi height increased ($P < 0.05$) on d 3, 7, 14, and 21 in prebiotic-treated birds compared with control birds reared in a hypoxic environment (Table 3). Birds fed a prebiotic diet and reared in a hypoxic environment had an average duodenum villi height of 17.21 ± 1.43 μm compared with 12.82 ± 1.26 μm in the hypoxic control diet birds on d 3. Similar effects were observed on d 7 when prebiotic-fed birds presented an average duodenum villi height of 22.83 ± 0.82 μm compared with 18.55 ± 0.93 μm in the control diet in broilers reared at simulated high altitudes. Duodenum villi height was not significantly different for diet treatments at the local altitude (Table 3). On d 14 and 21 control birds reared in a hypobaric chamber demonstrated reduced ($P < 0.05$) villi height compared with local altitude treatments; however, the Prebiotic-AM group housed in the hypobaric chamber was not different from the local altitude Prebiotic-AM group (Table 3).

Duodenum Villi Surface Area

The duodenum villi surface area was increased ($P < 0.05$) by feeding Prebiotic-AM compared with the control diet on d 7, 14, and 21 (Figure 1) in broilers reared in a hypoxic environment. Duodenum surface area was increased ($P < 0.05$) 61, 96, and 64% by the prebiotic treatment compared with the hypoxic controls on d 7, 14, and 21, respectively. On d 1 and 3 duodenum surface area was not significantly increased by either feed treatment at a local or a high altitude (Figure 1).

Duodenum Crypt Depth

Duodenum crypt depth was increased ($P < 0.05$) in Prebiotic-AM-treated birds compared with control birds

[4]MediaCybernetics, Silver Spring, MD.

TABLE 2. Effect of altitude and prebiotic on body weight and gut weight of commercial chicks reared at a local altitude or in a hypoxic environment[1]

	Sea level			Hypoxic		
Treatment	Body weight (g)	Gut weight (g)	Ratio[2]	Body weight (g)	Gut weight (g)	Ratio
Day 1						
Control	47.75 ± 0.75^{a,x}	6.43 ± 0.43^{a,x}	0.13	44.50 ± 1.85^{a,x}	5.56 ± 0.35^{a,x}	0.12
Prebiotic	43.00 ± 3.94^{a,x}	5.62 ± 0.98^{a,x}	0.13	45.50 ± 2.96^{a,x}	6.07 ± 0.52^{a,x}	0.13
Day 3						
Control	62.75 ± 2.50^{a,x}	8.82 ± 0.46^{a,x}	0.14	56.75 ± 2.50^{a,x}	6.93 ± 0.63^{a,x}	0.12
Prebiotic	62.50 ± 3.57^{a,x}	9.15 ± 0.96^{a,x}	0.15	59.75 ± 4.19^{a,x}	8.57 ± 0.93^{a,x}	0.14
Day 7 (wk 1)						
Control	91.00 ± 18.53^{a,x}	10.63 ± 3.11^{a,x}	0.11	105.75 ± 5.30^{a,x}	14.29 ± 0.73^{a,x}	0.14
Prebiotic	120.67 ± 13.12^{a,x}	14.26 ± 2.65^{a,x}	0.12	94.67 ± 5.36^{a,x}	11.60 ± 0.98^{a,x}	0.12
Day 14 (wk 2)						
Control	358.75 ± 42.50^{a,x}	38.17 ± 3.78^{a,x}	0.11	265.00 ± 30.04^{a,x}	25.86 ± 2.02^{a,y}	0.10
Prebiotic	328.33 ± 18.35^{a,x}	36.38 ± 8.07^{a,x}	0.11	262.75 ± 3.14^{a,y}	28.41 ± 1.97^{a,x}	0.09
Day 21 (wk 3)						
Control	708.00 ± 61.99^{a,x}	58.78 ± 5.04^{a,x}	0.08	557.50 ± 7.90^{a,y}	47.23 ± 4.26^{a,x}	0.08
Prebiotic	641.20 ± 67.65^{a,x}	56.98 ± 6.05^{a,x}	0.09	390.50 ± 43.99^{b,y}	28.51 ± 5.15^{b,y}	0.07
Day 28 (wk 4)						
Control	1,109.00 ± 53.03^{a,x}	78.52 ± 8.00^{a,x}	0.07	861.00 ± 75.55^{a,y}	57.88 ± 3.68^{a,x}	0.08
Prebiotic	1,100.30 ± 5.76^{a,x}	76.76 ± 9.40^{a,x}	0.07	713.30 ± 117.78^{a,x}	55.37 ± 11.93^{a,x}	0.07
Day 35 (wk 5)						
Control	1,841.30 ± 67.94^{a,x}	124.48 ± 7.81^{a,x}	0.07	1,156.00 ± 189.00^{a,,y}	42.61 ± 8.49^{a,,y}	0.04
Prebiotic	1,743.80 ± 115.16^{a,x}	121.71 ± 7.80^{a,x}	0.07	1,052.50 ± 9.50^{a,x}	51.28 ± 5.04^{a,,y}	0.05
Day 42 (wk 6)						
Control	2,399.50 ± 276.50^{a,x}	115.26 ± 6.58^{a,x}	0.05	1,521.00 ± 31.00^{a,x}	78.50 ± 15.40^{a,x}	0.05
Prebiotic	2,141.25 ± 108.27^{a,x}	93.19 ± 6.30^{a,x}	0.06	1,474.75 ± 193.36^{a,y}	79.75 ± 15.87^{a,y}	0.05

^{a,b}Significant ($P < 0.05$) between treatments within altitudes (vertical).

^{x,y}Significant ($P < 0.05$) within treatments between altitudes (horizontal).

[1]Values are means ± SEM representing 4 birds per group.

[2]Gut ratio was calculated as gut weight divided by body weight.

on d 1, 7, and 14 post hatch in the simulated high altitude environment (Table 4). Day 7 birds fed prebiotic (7.02 ± 0.35 μm) had deeper ($P < 0.05$) duodenum crypt depths when compared with controls (4.64 ± 0.25 μm) reared in the simulated high altitude environment. On d 14 the duodenum crypt depth was higher ($P < 0.05$, 72%) in prebiotic-treated birds compared with controls raised in the same hypoxic conditions. Similar to duodenum villi height and surface area in the Prebiotic-AM birds, the crypt depth of Prebiotic-AM group reared in the hypobaric chamber was not different from the local altitude Prebiotic-AM group.

TABLE 3. Effect of altitude and prebiotic on duodenum villi height of commercial chicks reared at a local altitude or in a hypoxic environment[1]

Treatment	Local (um)	Hypoxic (um)
Day 1		
Control	12.90 ± 0.74^{a,x}	11.44 ± 1.57^{a,x}
Prebiotic	11.39 ± 1.51^{a,x}	12.18 ± 2.06^{a,x}
Day 3		
Control	14.27 ± 2.74^{a,x}	12.82 ± 1.26^{b,x}
Prebiotic	14.63 ± 2.16^{a,x}	17.21 ± 1.43^{a,x}
Day 7		
Control	21.03 ± 3.99^{a,x}	18.55 ± 0.93^{b,x}
Prebiotic	26.97 ± 2.11^{a,x}	22.83 ± 0.82^{a,x}
Day 14		
Control	32.45 ± 1.12^{a,x}	25.68 ± 1.54^{b,y}
Prebiotic	35.39 ± 2.21^{a,x}	35.36 ± 1.03^{a,x}
Day 21		
Control	43.74 ± 3.44^{a,x}	28.09 ± 0.84^{b,y}
Prebiotic	46.15 ± 2.26^{a,x}	37.74 ± 2.60^{a,x}

^{a,b}Significant ($P < 0.05$) between treatments within altitudes (vertical).

^{x,y}Significant ($P < 0.05$) within treatments between altitudes (horizontal).

[1]Values are means ± SEM representing 4 birds per group and 14 measurments per parameter per bird.

Duodenum Lamina Propria Thickness

Duodenum lamina propria thickness was increased ($P < 0.05$) in prebiotic-treated birds reared at a local altitude on d 3 and 7 when compared with local altitude controls. Similar increases ($P < 0.05$) were observed in prebiotic-treated birds reared at a simulated high altitude on d 14 and 21 when compared with controls reared under the same conditions (Table 5).

Ileum Villi Height

The only differences observed for ileum villi height occurred on d 3 (Table 6). The ileum villi length was longer ($P < 0.05$) in prebiotic-treated birds compared with control birds reared in hypoxic environments on d 3 (Table 6). Ileum villi height was lower ($P < 0.05$) for the control birds reared in the hypobaric chamber on d 3 compared with the local altitude controls.

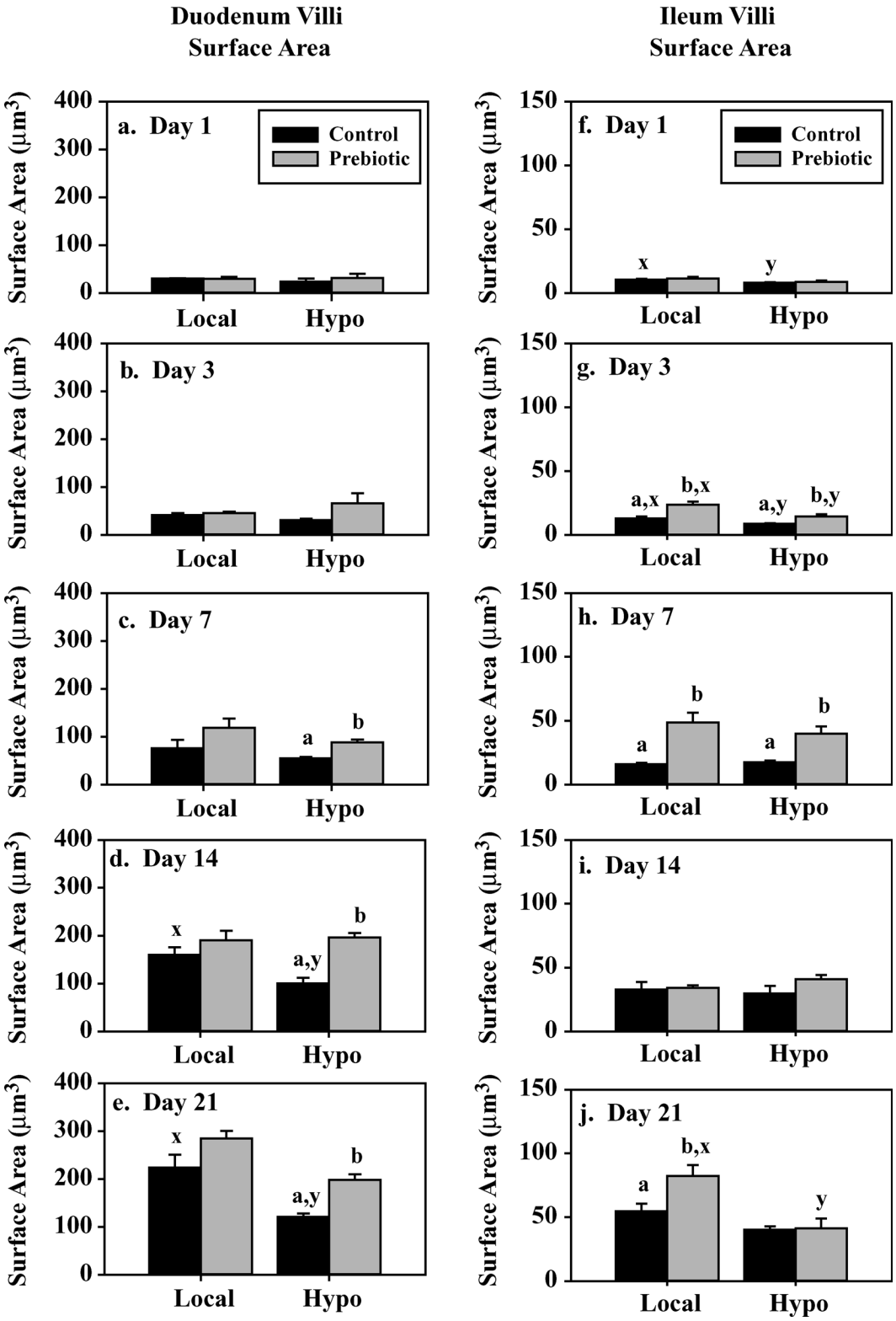

FIGURE 1. Average villi surface area of the duodenum and lower ileum of each group. Chicks were reared (n = 80) at a local altitude of 1,300 ft above sea level or a simulated high altitude of 9,500 ft above sea level to evaluate gut morphology. The groups were further divided into a total of 4 groups that were fed a control feed ration or an *Aspergillus* meal (0.2%) supplemented feed ration. Birds were euthanized on d 1, 3, 7, 14, and 21, and tissues from the duodenum and lower ileum were collected for histological evaluation. [a,b]Values with different letters indicate significant ($P < 0.05$) differences between treatments within an altitude treatment. [x,y]Values with different letters indicate significant ($P < 0.05$) differences within treatments between altitudes.

TABLE 4. Effect of altitude and prebiotic on duodenum crypt depth of commercial chicks reared at a local altitude or in a hypoxic environment[1]

Treatment	Local (um)	Hypoxic (um)
Day 1		
Control	3.75 ± 0.30[a,x]	3.45 ± 0.13[b,x]
Prebiotic	3.68 ± 0.21[a,x]	4.48 ± 0.34[a,x]
Day 3		
Control	5.68 ± 0.71[a,x]	5.58 ± 0.48[b,x]
Prebiotic	6.95 ± 0.78[a,x]	6.98 ± 1.33[a,x]
Day 7		
Control	7.88 ± 0.62[a,x]	4.64 ± 0.25[b,y]
Prebiotic	6.40 ± 0.73[a,x]	7.02 ± 0.35[a,x]
Day 14		
Control	6.88 ± 0.41[a,x]	4.95 ± 0.12[b,y]
Prebiotic	7.10 ± 0.25[a,x]	8.54 ± 0.57[a,x]
Day 21		
Control	5.96 ± 0.27[a,x]	6.78 ± 0.46[a,x]
Prebiotic	6.85 ± 0.37[a,x]	6.91 ± 0.41[a,x]

[a,b]Significant ($P < 0.05$) between treatments within altitudes (vertical).

[x,y]Significant ($P < 0.05$) within treatments between altitudes (horizontal).

[1]Values are means ± SEM representing 4 birds per group and 14 measurments per parameter per bird.

TABLE 6. Effect of altitude and prebiotic on ileum villi height of commercial chicks reared at a local altitude or in a hypoxic environment[1]

Treatment	Local (um)	Hypoxic (um)
Day 1		
Control	4.17 ± 0.18[a,x]	4.46 ± 0.41[a,x]
Prebiotic	5.40 ± 0.61[a,x]	5.28 ± 0.62[a,x]
Day 3		
Control	5.14 ± 0.30[a,x]	4.01 ± 0.12[a,y]
Prebiotic	6.04 ± 0.82[a,x]	5.08 ± 0.19[b,x]
Day 7		
Control	7.57 ± 0.91[a,x]	7.54 ± 0.93[a,x]
Prebiotic	10.74 ± 1.38[a,x]	9.60 ± 0.56[a,x]
Day 14		
Control	9.40 ± 0.56[a,x]	9.11 ± 1.46[a,x]
Prebiotic	8.10 ± 0.20[a,x]	10.24 ± 1.47[a,x]
Day 21		
Control	13.55 ± 1.06[a,x]	9.70 ± 1.01[a,y]
Prebiotic	14.68 ± 1.14[a,x]	9.93 ± 1.25[a,y]

[a,b]Significant ($P < 0.05$) between treatments within altitudes (vertical).

[x,y]Significant ($P < 0.05$) within treatments between altitudes (horizontal).

[1]Values are means ± SEM representing 4 birds per group and 14 measurments per parameter per bird.

Ileum Villi Surface Area

Ileum villi surface area was increased ($P < 0.05$) in Prebiotic-AM-treated birds compared with control birds on d 3 and 7 in both altitudes and on d 21 in birds housed in the local altitude battery (Figure 1). The control diet groups reared at a local elevation had an average ileum villi surface area of 12.97 ± 1.44 μm and 15.98 ± 0.88 μm, whereas the prebiotic treatment birds had ileum surface areas of 23.71 ± 2.51 μm and 48.63 ± 7.56 μm on d 3 and d 7, respectively. Similar results were observed within the simulated high altitude groups in which the control diet birds had surface areas of 8.76 ± 0.49 μm and 17.48 ± 1.38 μm compared with 14.44 ± 1.82 μm and 39.70 ± 5.80 μm in prebiotic-treated birds on d 3 and 7, respectively. The ileum surface area was reduced ($P < 0.05$) in the simulated high altitude compared with a local altitude on d 3 within each diet treatment (Figure 1).

Ileum Crypt Depth

Differences in ileum crypt depth were observed in d 7 and 21 samples. Ileum crypt depth was enhanced ($P < 0.05$) in the prebiotic supplemented birds compared with the control diet in both altitudes on d 7 (Table 7). Day 7 birds reared at a local altitude had an ileum crypt depth of 3.41 ± 0.32 μm in the control group and 6.66 ± 0.80 μm in the prebiotic-treated birds. Day 7 hypoxic control

TABLE 5. Effect of altitude and prebiotic on duodenum lamina propria thickness of commercial chicks reared at a local altitude or in a hypoxic environment[1]

Treatment	Local (um)	Hypoxic (um)
Day 1		
Control	2.20 ± 0.05[a,x]	2.25 ± 0.14[a,x]
Prebiotic	2.43 ± 0.13[a,x]	2.27 ± 0.17[a,x]
Day 3		
Control	2.97 ± 0.34[b,x]	3.06 ± 0.09[a,x]
Prebiotic	4.16 ± 0.20[a,x]	3.32 ± 0.02[a,x]
Day 7		
Control	3.41 ± 0.31[b,x]	3.52 ± 0.23[a,x]
Prebiotic	5.42 ± 0.19[a,x]	4.66 ± 0.46[a,x]
Day 14		
Control	4.40 ± 0.29[a,x]	3.95 ± 0.22[b,x]
Prebiotic	4.34 ± 0.30[a,x]	5.22 ± 0.24[a,x]
Day 21		
Control	5.38 ± 0.20[a,x]	4.40 ± 0.06[b,y]
Prebiotic	5.26 ± 0.18[a,x]	5.27 ± 0.21[a,x]

[a,b]Significant ($P < 0.05$) between treatments within altitudes (vertical).

[x,y]Significant ($P < 0.05$) within treatments between altitudes (horizontal).

[1]Values are means ± SEM representing 4 birds per group and 14 measurments per parameter per bird.

TABLE 7. Effect of altitude and prebiotic on ileum crypt depth of commercial chicks reared at a local altitude or in a hypoxic environment[1]

Treatment	Local (um)	Hypoxic (um)
Day 1		
Control	2.45 ± 0.06[a,x]	2.40 ± 0.14[a,x]
Prebiotic	2.38 ± 0.17[a,x]	2.78 ± 0.32[a,x]
Day 3		
Control	2.23 ± 0.34[a,x]	2.35 ± 0.12[a,x]
Prebiotic	4.01 ± 0.40[a,x]	2.95 ± 0.15[a,x]
Day 7		
Control	3.41 ± 0.32[b,x]	2.81 ± 0.15[b,x]
Prebiotic	6.66 ± 0.80[a,x]	6.04 ± 0.53[a,x]
Day 14		
Control	4.10 ± 0.22[a,x]	3.20 ± 0.46[b,y]
Prebiotic	3.79 ± 0.10[a,x]	4.66 ± 0.46[a,x]
Day 21		
Control	4.93 ± 0.42[a,x]	3.62 ± 0.13[a,y]
Prebiotic	5.43 ± 0.32[a,x]	4.15 ± 0.30[a,y]

[a,b]Significant ($P < 0.05$) between treatments within altitudes (vertical).

[x,y]Significant ($P < 0.05$) within treatments between altitudes (horizontal).

[1]Values are means ± SEM representing 4 birds per group and 14 measurments per parameter per bird.

TABLE 8. Effect of altitude and prebiotic on ileum lamina propria thickness of commercial chicks reared at a local altitude or in a hypoxic environment[1]

Treatment	Local (um)	Hypoxic (um)
Day 1		
Control	1.78 ± 0.12^{a,x}	1.73 ± 0.17^{b,x}
Prebiotic	1.89 ± 0.17^{a,x}	1.98 ± 0.20^{a,x}
Day 3		
Control	1.91 ± 0.07^{b,x}	1.82 ± 0.15^{b,x}
Prebiotic	3.14 ± 0.31^{a,x}	2.35 ± 0.09^{a,x}
Day 7		
Control	2.20 ± 0.22^{b,x}	2.30 ± 0.22^{b,x}
Prebiotic	4.38 ± 0.45^{a,x}	4.45 ± 0.31^{a,x}
Day 14		
Control	2.91 ± 0.17^{a,x}	2.30 ± 0.38^{b,x}
Prebiotic	3.05 ± 0.29^{a,x}	3.57 ± 0.34^{a,x}
Day 21		
Control	4.56 ± 0.39^{a,x}	3.24 ± 0.12^{b,y}
Prebiotic	4.91 ± 0.21^{a,x}	3.95 ± 0.24^{a,y}

^{a,b}Significant ($P < 0.05$) between treatments within altitudes (vertical).

^{x,y}Significant ($P < 0.05$) within treatments between altitudes (horizontal).

[1]Values are means ± SEM representing 4 birds per group and 14 measurments per parameter per bird.

samples had crypt depths of 2.81 ± 0.15 μm compared with 6.04 ± 0.53 μm for prebiotic birds reared in the hypobaric chamber. Hypoxia decreased ($P < 0.05$) ileum crypt depths on d 21 within feed treatments from 4.93 ± 0.42 μm in the local altitude group to 3.62 ± 0.13 μm in the simulated high altitude group fed control diets and from 5.43 ± 0.32 μm to 4.15 ± 0.30 μm in local altitude and high altitude Prebiotic-AM groups, respectively.

Ileum Lamina Propria Thickness

Ileum lamina propria thickness was greater ($P < 0.05$) in the Prebiotic-AM group on d 3 and 7 when compared with controls reared in the local altitude environment (Table 8). The prebiotic-treated bird's lamina propria thickness was greater on d 3, 7, 14, and 21 when compared with controls reared in the hypoxic environment ($P < 0.05$). The only differences ($P < 0.05$) observed in lamina propria thickness within treatments at different altitudes occurred on d 21 in birds fed the control and Prebiotic-AM diet (Table 8).

DISCUSSION

Broilers fed Prebiotic-AM and reared under hypoxic conditions had 23% lower incidence of ascites compared with control birds raised in the same environment over the course of the study. As birds matured, body and gut weights were lower in hypoxic conditions compared with local altitude for control and prebiotic supplemented birds. In hypoxic conditions, body and gut weights were lower on d 21 in Prebiotic-AM-treated birds and were numerically lower in wk 4 to 6 compared with controls. Lower body weights might have contributed to the reduced incidence of ascites observed, as reducing growth rates is a method of controlling ascites (Balog, 2003). The reduction of ascites incidence in the prebiotic-treated birds might also have been associated with increased gut efficiency by stimulating early gut maturation (Uni and Ferket, 2004), thereby reducing the negative effects of hypoxia, which can lead to ascites. Gut maturation was significantly reduced in hypoxic conditions when compared with local altitude birds for several parameters, but it is also important to note that prebiotic supplementation allowed birds in hypoxic conditions to maintain gastrointestinal maturation and development at rates similar to local altitude birds. Early maturation of the gut has been shown to be an important factor in raising a healthy chick, as the physiological development of birds is directly related to digestion and nutrient absorption in the small intestine (Aptekmann et al., 2001). In this study, significant increases in gut development were observed 3 d post hatch, as previously reported by Uni and coworkers (1999). These dramatic developmental changes occur in the avian gut 2 to 3 d post hatch due to a change in nutrient sources from yolk to an exogenous feed ration rich in carbohydrates (Uni et al., 1999).

Recent studies conducted in our laboratories suggest that prebiotics, such as *Aspergillus* meal, can enhance the gastrointestinal maturation of broiler chicks (unpublished data). Prebiotics selectively modify beneficial bacteria of the small intestine, potentially influencing gut metabolism (Walker and Duffy, 1998) and increasing resistance to pathogenic microorganisms (Manning and Gibson, 2004). *Aspergillus* meal is derived from the active fermentation of *Aspergillus oryzae*, which has been shown to enhance gut development and nutrient digestibility by increasing beneficial gut microflora and short chain fatty acids (Gomez-Alarcon et al., 1990; Yanahira et al., 1995; Yoon and Stern, 1996; Beharka and Nagaraja, 1998; Hirayama et al, 2000). Bacteria are an important part of gut development, as gnotobiotic animals have immature GIT with reduced nutrient absorption, decreased immune function, and limited pathogen resistance at hatch or birth (Farthing, 2004). Beneficial microflora promote gut health by influencing enterocyte turnover, competing with pathogenic bacteria for nutrients and binding sites, and producing bacteriostatic compounds that limit the growth of pathogenic bacteria (Farthing, 2004). *Lactobacillus* and *Bifidobacterium* are examples of these beneficial bacteria that proliferate in broilers fed a diet supplemented with a prebiotic (Manning and Gibson, 2004).

Prebiotic supplementation appeared to influence several gut parameters in our study. We observed increases in duodenum and ileum villi heights after 3 d post hatch in the Prebiotic-AM-treated birds reared at a simulated high altitude compared with environmental controls; however, the largest increases were observed in the duodenum. Duodenum villi height was significantly increased in prebiotic-treated birds housed in the hypobaric chamber on d 3, 7, 14, and 21 by 34, 23, 38, and 34%, respectively, when compared with simulated high altitude controls. Ileum villi height was also increased by 27% on d 3 in prebiotic birds housed in a hypoxic environment compared with controls reared in the same environment.

The villi play a crucial role in the digestion and absorption processes of the small intestine, as villi increase surface area and are the first to make contact with nutrients in the lumen (Gartner and Hiatt, 2001). Below the surface of the intestinal villi are capillaries that absorb the digested products of carbohydrates and proteins. Fats are broken down and absorbed across the intestinal wall into the lacteals deeper inside the villi (Advance Online Nutrition, 2003). Researchers have previously demonstrated significant increases in villi height by supplementing poultry diets with calcium (Aptekmann et al., 2001). The increased duodenum villi height observed in our study has been previously reported in turkey poults (Noy et al., 2001) and may be explained by the enhanced efficiency of digestion and absorption of the duodenum due to a population of beneficial bacteria that supply nutrients and stimulate vascularization and development of intestinal villi (Bedford, 2000b; Gilmore and Ferretti, 2003).

Lamina propria thickness of the duodenum was significantly increased in Prebiotic-AM-treated birds compared with controls reared at a local altitude on d 3 and 7. Similar results were observed at a simulated high altitude on d 14 and 21. These data indicate that Prebiotic-AM may immunomodulate the GIT of broilers. Only a layer of mucin and a single cell layer of enterocytes separate the deeper tissues of the body from potential opportunistic pathogens found in the lumen of the GIT (Macpherson and Harris, 2004). The lamina propria contains connective tissue in the mucosa that supports the delicate enterocytes of the villi and is an essential component of the immune system, as a thin or scattered lamina propria is more readily infiltrated by pathogens (Gartner and Hiatt, 2001). Lamina propria thickness can be used as an indicator of gut health because it contains dendritic cells that survey the contents of the lumen and protect the chicken from infection by stimulating the adaptive immune response, increasing gut motility, and modifying mucin production, defensin secretion, and IgA production (Macpherson and Harris, 2004).

The crypts of the villus contain several specialized cells such as absorptive cells, goblet cells, and regenerative cells that are responsible for the production of mucus and the replacement of old cells. We observed significant increases in duodenum and ileum crypt depth in birds fed prebiotic on d 3 and 7, with the greatest difference observed on d 7. On d 7, prebiotic-fed birds had a duodenum crypt depth that was increased by 51% in birds reared in a hypoxic environment, by 42% in the ileum crypts of birds raised at a local altitude, and by 42% in birds reared in a hypoxic environment when compared with control-fed birds reared in similar environments. One reason for the improvement in crypt depth observed in prebiotic treated birds may be due to the rapid crypt maturation that occurs in chicks during the first week of life (Geyra et al., 2001).

In conclusion, these studies show that ascites incidence was significantly reduced in the Prebiotic-AM treated birds when compared with controls housed in the same hypoxic conditions. It is also important to note that the hypoxic Prebiotic-AM group had improved or similar levels of gut development as the local altitude controls, which suggests that the Prebiotic-AM treatment may negate the effect of hypoxia in broilers. This report is the first to relate gut development to ascites in broilers. The data suggest that *Aspergillus* meal may reduce ascites in commercial broilers by enhancing gut development allowing birds to use more oxygen and maintain their current high metabolism.

ACKNOWLEDGMENT

The authors thank Sonia Tsai (Poultry Production and Product Safety Research Unit) and Kinnari Pandya (University of Arkansas) for their help with the sample collection and statistical analysis and Wally McDonner (Poultry Production and Product Safety Research Unit) for his help with sample collection and operating and maintaining the hypobaric chamber. We acknowledge the Fulbright Visiting Scholar Program for funding Fausto Solis de los Santos (University of Arkansas) and Lewis M. Sutton (PetAg Inc., Hampshire, IL) for partial support of this study.

REFERENCES

Advance Online Nutrition. 2003. Module 2.2: Digestion and absorption in the small intestine. www.speedyvet.com/nutrition. Accessed Nov. 2004.

Apajalahti, J., A. Kettunen, and H. Graham. 2004. Characteristics of the gastrointestinal microbial communities with special reference to the chicken. Worlds Poult. Sci. J. 60:223–232.

Aptekmann, K. P., S. M. Baraldi Arton, M. A. Stefanini, and M. A. Orsi. 2001. Morphometric analysis of the intestine of domestic quails (*Coturnix coturnix japonica*) treated with different levels of dietary calcium. Anat. Histol. Embryol. 30:277–280.

Balog, J. M., N. B. Anthony, M. A. Cooper, B. D. Kidd, G. R. Huff, W. E. Huff, and N. C. Rath. 2000. Ascites syndrome and related pathologies in feed restricted broilers raised in a hypobaric chamber. Poult. Sci. 79:318–323.

Balog, J. M. 2003. Ascites syndrome (pulmonary hypertension syndrome) in broiler chickens: Are we seeing the light at the end of the tunnel? Avian Poult. Biol. Rev. 14:99–126.

Balog, J. M., B. D. Kidd, W. E. Huff, G. R. Huff, N. C. Rath, and N. B. Anthony. 2003. Effect of cold stress on broilers selected for resistance or susceptibility to ascites. Poult. Sci. 82:1383–1387.

Bedford, M. 2000a. Exogenous enzymes in monogastric nutrition-their current value and future benefits. Anim. Feed Sci. Technol. 86:1–13.

Bedford, M. 2000b. Removal of antibiotic growth promoters from poultry diets: Implications and strategies to minimize subsequent problems. Worlds Poult. Sci. J. 56:347–365.

Beharka, A. A., and T. G. Nagaraja. 1998. Effect of *Aspergillus oryzae* extract alone or in combination with antimicrobial compounds on ruminal bacteria. J. Dairy Sci. 81:1591–1598.

Duncan, D. B. 1955. Multiple range and multiple F tests. Biometrics 11:1–42

Farthing, M. J. G. 2004. Bugs and the gut: An unstable marriage. Best Pract. Res. Clin. Gastroenterol. 18:233–239.

Furese, M., S. I. Yang, N. Niwa, and J. Okumura. 1991. Effect of short chain fatty acids on the performance and the intestinal weight in germ free and conventional chicks. Br. Poult. Sci. 32:159–165.

Gartner, L. P., and J. L. Hiatt. 2001. Color Textbook of Histology. 2nd ed. W. B. Saunders, Baltimore, MD.
Geyra, A., Z. Uni, and D. Sklan. 2001. Enterocyte dynamics and mucosal development in the posthatch chick. Poult. Sci. 80:776–782.
Gibson, G. R., E. Beatty, X. Wang, and J. Cummings. 1995. Selective stimulation of *Bifidobacteria* in the human colon by oligofructose and inulin. Gastroenterology 108:975–982.
Gilmore, M. S., and J. J. Ferretti. 2003. The thin line between gut commensal and pathogen. Science 299:1999–2002.
Gomez-Alacon, R. A., C. Dudas, and J. T. Huber. 1990. Influence of cultures of *Aspergillus oryzae* on rumen and total tract digestibility of dietary components. J. Dairy Sci. 73:703–710.
Havenstein, G. B., P. R. Ferket, S. E. Scheideler, and B. T. Larson. 1994. Growth, livability, and feed conversion of 1957 vs. 1991 broilers when fed "typical" 1957 and 1991 broiler diets. Poult. Sci. 73:1785–1794.
Hirayama, F., T. Ogata, H. Yano, H. Arima, K. Udo, M. Takano, and K. Uekama. 2000. Release characteristics of a short-chain fatty acid, n-butyric acid, from its beta-cyclodextrin ester conjugate in rat biological media. J. Pharm. Sci. 89:1486–1495.
Ivatury, R. R., R. J. Simon, S. Islam, A. Fueg, M. Rohman, and W. M. Stahl. 1996. A prospective randomized study of end points of resuscitation after major trauma: Global oxygen transport indices versus organ-specific gastric mucosal pH. J. Am. Coll. Surg. 183:145–154.
Julian, R. J. 2000. Physiological, management and environmental triggers of the ascites syndrome: A review. Avian Pathol. 29:519–527.
Macpherson, A. J., and N. L. Harris. 2004. Interactions between commensal intestinal bacteria and the immune system. Nat. Rev. Immunol. 4:478–485.
Manning, T. S., and G. R. Gibson. 2004. Prebiotics. Best Pract. Res. Clin. Gastroenterol. 18:287–298.
National Research Council. 1994. Nutrient Requirements of Poultry. 9th rev. ed. National Academy Press, Washington DC.
Noy, Y., A. Geyra, and D. Sklan. 2001. The effect of early feeding on growth and small intestinal development in the posthatch poult. Poult. Sci. 80:912–919.
Owen, R. L., R. F. Wideman, A. L. Hattel, and B. S. Cowen. 1990. Use of a hypobaric chamber as a model system for investigating ascites in broilers. Avian Dis. 34:754–758.
Pakdel, A., J. A. Van Arendonk, A. L. Vereijken, and H. Bovenhuis. 2002. Direct and maternal genetic effects for ascites-related traits in broilers. Poult. Sci. 81:1273–1279.
Palmer, M. F., and B. A. Rolls. 1983. The activities of some metabolic enzymes in the intestines of germ free and conventional chicks. Br. J. Nutr. 50:783–790.
Riddell, C. 1991. Developmental, metabolic, and miscellaneous disorders. Pages 839–841 in Diseases of Poultry. 9th ed. Iowa State University Press. Ames, IA.
Sakamoto, K., H. Hirose, A. Onizuka, M. Hayashi, N. Futamura, Y. Kawamura, and T. Ezaki. 2000. Quantitative study of changes in intestinal morphology and mucus gel on total parenteral nutrition in rats. J. Surg. Res. 94:99–106.
SAS Institute. 1988. SAS/STAT User's Guide, Release 6.03 ed. SAS Institute Inc., Cary, NC.
Uni, Z., and R. P. Ferket. 2004. Methods for early nutrition and their potential. Worlds Poult. Sci. J. 60:101–111.
Uni, Z., Y. Noy, and D. Sklan. 1999. Posthatch development of small intestinal function in the poult. Poult. Sci. 78:215–222.
Uni, Z., E. Tako, O. Gal-Garber, and D. Sklan. 2003. Morphological, molecular, and functional changes in the chicken small intestine of the late-term embryo. Poult. Sci. 82:1747–1754.
Walker, W. A., and L. C. Duffy. 1998. Diet and bacterial colonization: Role of probiotics and prebiotics. J. Nutr. Biochem. 9:668–675.
Wideman, R. F., Jr., D. M. Hooge, and K. R. Cummings. 2003. Dietary sodium bicarbonate, cool temperatures, and feed withdrawal: Impact on arterial and venous blood-gas values in broilers. Poult. Sci. 82:560–570.
Yanahira, S., M. Morita, S. Aoe, T. Suguri, I. Nakajima, and E. Deya. 1995. Effects of lactitol-oligosaccharides on the intestinal microflora in rats. J. Nutr. Sci. Vitaminol. 41:83–94.
Yen, J. T., J. A. Nienaber, D. A. Hill, and W. G. Pond. 1989. Oxygen consumption by portal vein-drained organs and by whole animal in conscious growing swine. Proc. Soc. Exp. Biol. Med. 190:393–398.
Yokota, H., and M. E. Coates. 1982. The uptake of nutrients from the small intestine of gnotobiotic and conventional chicks. Br. J. Nutr. 47:349–356.
Yoon, I. K. and M.D. Stern. 1996. Effects of *Saccharomyces cerevisiae* and *Aspergillus oryzae* cultures on ruminal fermentation in dairy cows. J. Dairy Sci. 79:411–417.